

WHAT IS CLAIMED IS:

*Sub 1*  
1. A formulation comprising Apo-2 ligand and one or more divalent metal ions, wherein the concentration of said one or more divalent metal ions present in the formulation is at a  $<2\times$  molar ratio to said Apo-2 ligand.

2. The formulation of claim 1 wherein said one or more divalent metal ions comprises zinc or cobalt.

3. The formulation of claim 2 wherein said one or more divalent ions comprises zinc.

4. The formulation of claim 3 wherein said zinc is selected from the group consisting of zinc chloride, zinc acetate, zinc sulfate, zinc carbonate and zinc citrate.

5. The formulation of claim 1 wherein said formulation is a pharmaceutically acceptable formulation.

6. The formulation of claim 1 wherein said Apo-2 ligand comprises amino acids 114 to 281 of Figure 1 (SEQ ID NO:1).

*Sub 2*  
7. The formulation of claim 1 wherein said Apo-2 ligand comprises amino acids 1 to 281 of Figure 1 (SEQ ID NO:1) or a biologically active fragment or variant thereof.

8. The formulation of claim 1 wherein said formulation has a pH of about 6 to about 9.

9. The formulation of claim 8 wherein said formulation has a pH of about 7 to about 7.5.

10. The formulation of claim 1 wherein said formulation is an aqueous formulation.

11. The formulation of claim 1 wherein said formulation is a lyophilized formulation.

12. A formulation comprising Apo-2 ligand and one or more divalent metal ions, wherein the concentration of said one or more divalent metal ions present in the formulation is at a  $\geq 2X$  molar ratio to said Apo-2 ligand.

13. A method of enhancing formation of Apo-2 ligand trimers, comprising exposing Apo-2 ligand polypeptides to an effective amount of one or more divalent metal ions.

14. A method of making a pharmaceutically acceptable formulation of Apo-2 ligand, comprising admixing Apo-2 ligand, an effective amount of one or more divalent metal ions, and a pharmaceutically acceptable carrier.

15. A method of reducing formation of disulfide-linked Apo-2 ligand dimers, comprising exposing Apo-2 ligand polypeptides to an effective amount of one or more divalent metal ions.

16. A method of making Apo-2 ligand, comprising the steps of: (a) providing a host cell comprising a replicable vector containing a nucleotide sequence encoding Apo-2 ligand polypeptide; (b) providing culture media containing an effective amount of one or more divalent metal ions; (c) culturing the host cell in the culture media under conditions sufficient to express the Apo-2 ligand; and (d) recovering the Apo-2 ligand from the host cell or culture media.

17. The method of claim 16 wherein said host cell is *E. coli*.

18. The method of claim 16 wherein said one or more divalent metal ions comprises zinc.

19. The method of claim 18 wherein said zinc comprises zinc sulfate.

20. The method of claim 16 wherein said one or more divalent metal ions comprises cobalt.

21. The method of claim 20 wherein said cobalt comprises cobalt chloride.

22. The method of claim 18 wherein said zinc is present in the culture media at a concentration of about 50 micromolar to about 250 micromolar.

23. The method of claim 16 wherein said replicable vector comprises a nucleotide sequence encoding one or more tRNA molecules.

24. The method of claim 23 wherein said replicable vector is the pAPAp2-P2RU vector.

25. The method of claim 16 wherein said Apo-2 ligand comprises amino acids 114 to 281 of Figure 1 (SEQ ID NO:1).

26. The method of claim 16 wherein said Apo-2 ligand comprises amino acids 1 to 281 of Figure 1 (SEQ ID NO:1) or a biologically active fragment or variant thereof.

27. A method of making Apo-2 ligand, comprising the steps of: providing a host cell comprising a replicable vector containing a nucleotide sequence encoding Apo-2 ligand; (b) providing culture media; (c) culturing the host cell in the culture media under conditions sufficient to express the Apo-2 ligand; (d) recovering the Apo-2 ligand from the host cell or culture media; and (e) purifying the Apo-2 ligand in the presence of an effective amount of one or more divalent metal ions.

28. The method of claim 27 wherein in step (e), said Apo-2 ligand is purified in the presence of one or more divalent metal ions and a reducing agent.

29. The method of claim 27 wherein said Apo-2 ligand comprises amino acids 114 to 281 of Figure 1 (SEQ ID NO:1).

30. The method of claim 27 wherein said Apo-2 ligand comprises amino acids 1 to 281 of Figure 1 (SEQ ID NO:1) or a biologically active fragment or variant thereof.

31. A method for recovering Apo-2 ligand from a prokaryotic cell culture comprising the steps of (a) isolating Apo-2 ligand which has been expressed in cultured prokaryote host cells; (b) exposing said isolated Apo-2 ligand to a buffered solution containing one or more divalent metal ions and reducing agent; and (c) recovering said isolated Apo-2 ligand.

32. The method of claim 31 wherein in step (b), said one or more divalent metal ions is selected from the group consisting of zinc and cobalt and said reducing agent is selected from the group consisting of DTT and BME.

33. The method of claim 31 wherein in step (c) said Apo-2 ligand is recovered by sequentially contacting said Apo-2 ligand to a cationic chromatography support, hydroxyapatite support, and hydrophobic chromatographic support.

34. The method of claim 33 wherein said cationic chromatography support is selected from the group consisting of SP-Sepharose, CM-Sepharose, and Macro-Prep ceramic HS resin.

35. The method of claim 33 wherein said hydrophobic chromatographic support is selected from the group consisting of phenyl agarose, butyl agarose, TSK resin, and Toyopearl resin.

36. An isolated Apo-2 ligand variant polypeptide comprising an amino acid sequence which differs from native sequence Apo-2 ligand and has one or more of the following amino acid substitutions at the residue position(s) in Figure 1 (SEQ ID NO:1): R130A; N134A; L136A; S138A; N140A; N143A; S153A; E155A; R158A; R170A; K179A; R191A; Q193A; E195A; K197D; K201A; N202A; D203A; Y213A; D218A; Y240A; K251A; S259A; D267A; D269A.

37. An isolated Apo-2 ligand variant polypeptide comprising an amino acid sequence which differs from native sequence Apo-2 ligand and has one or more of the following amino acid substitutions at the residue position(s) in Figure 1 (SEQ ID NO:1): S141A, K142A, S159A, H264A.

38. An isolated Apo-2 ligand variant polypeptide comprising an amino acid sequence which differs from native sequence Apo-2 ligand and has one or more of the following amino acid substitutions at the residue position(s) in Figure 1 (SEQ ID NO:1): R149A; C230S; C230A; Q205A; V207A; Y216A; E236A; Y237A.

39. An isolated nucleic acid comprising a nucleotide sequence encoding the Apo-2 ligand variant of claim 36.

40. A vector comprising the nucleic acid of claim 39.

41. A host cell comprising the vector of claim 40.

~~42.~~ The pAPAp2-P2RU vector.

43. A host cell comprising the vector of claim 42.

44. The host cell of claim 43 wherein said host cell is *E. coli*.

45. An isolated Apo-2 ligand comprising amino acids 114 to 281 of Figure 1 (SEQ ID NO:1) and made according to the method of claim 16.

46. An isolated Apo-2 ligand comprising amino acids 1 to 281 of Figure 1 (SEQ ID NO:1) or a biologically active fragment or variant thereof, and made according to the method of claim 16.

47. An isolated Apo-2 ligand comprising amino acids 114 to 281 of Figure 1 (SEQ ID NO:1) and made according to the method of claim 27.

48. An isolated Apo-2 ligand comprising amino acids 1 to 281 of Figure 1 (SEQ ID NO:1) or a biologically active fragment or variant thereof, and made according to the method of claim 27.

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